Finally, it is interesting to note that, like some other highly conserved ACRs (see, for example, [3]), YjeR family proteins cannot be strictly essential for cell function, as they are not encoded in the 'minimal' genomes of mycoplasmas, nor in the larger genomes of the cyanobacterium *Synechocystis* sp. and of three archaea.

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Photoactivation of green fluorescent protein

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Considerable insight into many cell biological processes can be obtained by following the turnover of individual protein species in time and space. In living cells, this has been achieved by following fluorescence recovery after local microbeam photobleaching of microinjected fluorescently labelled protein (see, for example, [1]) as well as by photoactivation of 'caged' fluorescently labelled proteins [2,3].

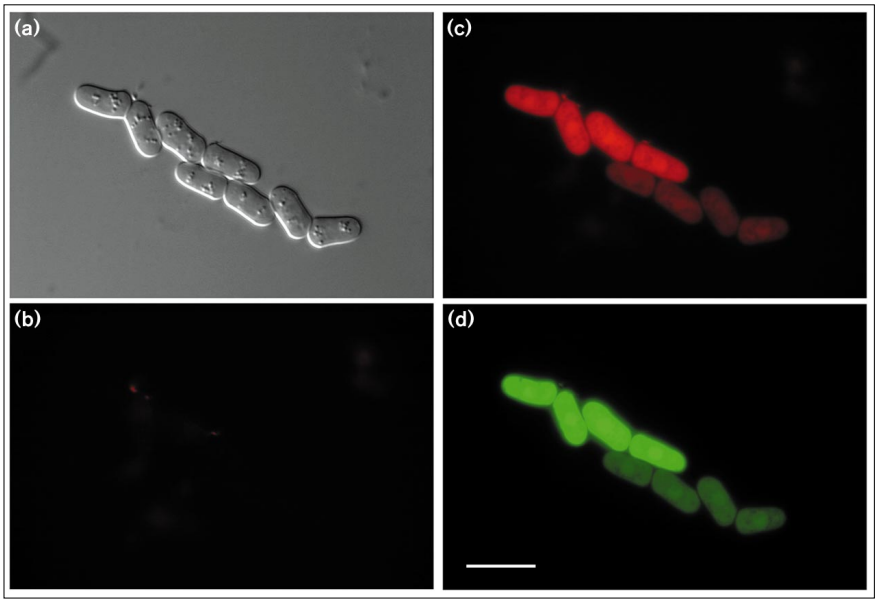
The gene encoding the naturally occurring green fluorescent protein (GFP) of the cnidarian *Aequorea victoria* [4] has proved invaluable as an *in vivo* fluorescence tag for the subcellular localization of proteins and/or as a reporter of specific promoter and/or enhancer activities in a multitude of organisms [5]. Recently, GFP has been used to study protein dynamics in cells using photobleaching [6,7] and local fluorescence enhancement [8]. Here, we describe *in vivo* photoactivation of GFP to a novel red fluorescent form by illumination with blue light. We hope this method will be of use and interest to many investigators.

Fission yeast (*Schizosaccharomyces pombe*) expressing the red-shifted variant GFPmut2 [9] in the multi-copy plasmid pSGA [10] were immobilized at low density on a thin pad of EMM2 minimal medium containing 2% agarose and sealed under a coverslip with paraffin wax. Under these conditions cells can divide for several generations at nearly normal rates. After 2–3 cell divisions we illuminated microcolonies of cells with blue light (fluorescein filter set; 460–500 nm excitation), observing red fluorescence (Texas Red filter set; 540–580 nm excitation, 610–680 nm emission) before and after blue illumination (Fig. 1).

We found that relatively short exposures to blue light (1–5 sec) are sufficient to generate a significant photoconversion of GFPmut2 to a stable red fluorescent form, and that the amount of red fluorescence produced is proportional to the total green fluorescence. When viewed with a Cy5 filter set (590–650 nm excitation, 670–730 nm emission), the signal was considerably more faint; with a tetramethyl rhodamine filter set (510–555 nm excitation, 570–640 nm emission), cells were already visible before photoactivation, but became brighter with increasing exposure. We have observed photoactivation with several different GFP fusion proteins, located in the cytoplasm and in the nucleus, and associated with the plasma membrane.

Although attempts to photoactivate cells taken directly from exponentially growing shaken cultures were unsuccessful, cells taken from a pellet after a brief (5–10 min) centrifugation were easily photoactivated, whether in minimal medium or in a phosphate-buffered saline solution. Photoactivatability was immediately lost on vigorous shaking of the centrifuged cells. Isolation from the atmosphere thus seems to be an important factor in photoactivation, consistent with additional observations that cells at

Figure 1



Photoactivation of GFP to a red-fluorescent protein on exposure to blue light. **(a)** DIC image of a microcolony of cells growing on an agarose pad in a sealed chamber. **(b)** Texas Red image of the same microcolony before photoactivation. **(c)** Texas Red image (same exposure time) after 2 sec photoactivation, using blue light from the fluorescein filter set. **(d)** Fluorescein image, at the end of the experiment. The bottom four cells are expressing less protein, probably because of plasmid mis-segregation during the first division. Note that photoactivated red fluorescence is proportional to green fluorescence, and that faint background fluorescence in the Texas Red channel (from out-of-focus debris) does not appear in the fluorescein channel. Bar is 10 μm. Images were collected on a Power Macintosh 8500 computer using a Hamamatsu C5985 chilled video-rate CCD mounted on a Zeiss Axiomat microscope (40×/0.7 Plan-Neofluar objective) equipped with High-Q filter sets (Chroma Technology, Brattleboro, Vermont). Exposure times were 0.04 sec for DIC, 2 sec for both Texas Red images, and 0.2 sec for the fluorescein image; no further image processing was applied.

the periphery of an unsealed chamber are poorly activated, whereas those in the center activate readily. One possible reason for this could be that some amount of oxygen depletion from the system may be required for efficient photoactivation; fission yeast grow completely normally under non-aerated, and thus near-anaerobic, conditions [11].

Although the mechanisms underlying photoactivation of GFP are not yet clear, photoactivation does not seem to be specific to GFPmut2, as we have observed similar effects with both wild-type GFP and the S65T mutant [12] — although wild-type GFP was photoactivated with ultraviolet light (340–380 nm) more efficiently than with blue light — in all cases only under the conditions described above.

Single-celled eukaryotes such as yeast have been for the most part ignored in studies of intracellular protein dynamics because they are recalcitrant to microinjection. Irrespective of the exact mechanisms involved in photoactivation, our observations demonstrate that photoactivating GFP into a red fluorescent protein will be a useful tool for cell biology, and we hope that these methods will be easily adaptable to other systems, including mammalian cells. Photoactivation of GFP fusion proteins would circumvent the need to microinject cells of all types and, in combination with local microbeaming of subcellular regions, should allow for interesting new insights into the dynamic behavior of protein assemblies within living cells.

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